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# An association between a variation in the *PSCA* gene and upper gastrointestinal cancer in Caucasians

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## Abstract

**Background & Aims—**An association between gastric cancer and the rs2294008 (C>T) polymorphism in the *prostate stem cell antigen* (*PSCA*) gene has been reported for several Asian populations. We set out to determine whether such an association exists in Caucasians.

**Methods—**We genotyped 166 relatives of gastric cancer patients, including 43 *H pylori*-infected subjects with hypochlorhydria and gastric atrophy, 65 infected subjects without these abnormalities, 58 *H pylori*-negative relatives, and 100 population controls. Additionally, a population-based study of chronic atrophic gastritis provided 533 cases and 1054 controls. We then genotyped 2 population-based case-control studies of upper gastrointestinal cancer: the first included 312 gastric cancer cases and 383 controls; the second included 309 gastric cancer cases,



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159 esophageal cancer cases, and 211 controls. Odds ratios were computed from logistic models and adjusted for confounding variables.

**Results—**Carriage of the risk allele (T) of rs2294008 in *PSCA* was associated with chronic atrophic gastritis (adjusted odds ratio [OR] = 1.5; 95% confidence interval [CI], 1.1–1.9) and non-cardia gastric cancer (OR = 1.9; 95% CI, 1.3– 2.8). The association was strongest for the diffuse histological-type (OR = 3.2; 95% CI, 1.2–10.7). An inverse association was observed between carriage of the risk allele and gastric cardia cancer (OR = 0.5; 95% CI, 0.3–0.9), esophageal adenocarcinoma (OR = 0.5; 95% CI, 0.3–0.9), and esophageal squamous cell carcinoma (OR = 0.4; 95% CI, 0.2–0.9).

**Conclusions—**The rs2294008 polymorphism in *PSCA* increases the risk of non-cardia gastric cancer and its precursors in Caucasians but protects against proximal cancers.

### Keywords

Stomach cancer; esophageal cancer; genetic polymorphisms, cancer genetics

## Introduction

Upper gastrointestinal cancers represent a significant global health burden. Gastric and esophageal cancers were, respectively, the second and sixth most common causes of cancer-related mortality worldwide in 2008, accounting for over one million deaths[1].

The identification of *Helicobacter pylori* as the major acquired etiological agent responsible for gastric carcinogenesis2,3 revolutionized our understanding of the inflammation and cancer paradigm, and prompted candidate gene approaches to host genetic susceptibility4,5. Consequently, gastric cancer is arguably better understood in terms of genetic susceptibility than other gastrointestinal malignancies. We, and other authors, have previously reported an increased risk of gastric cancer and its precursors (gastric atrophy and hypochlorhydria) in association with polymorphisms in pro-inflammatory cytokine genes (*IL-1B, IL-1RN, TNFA and IL-10*), and genes involved in the innate immune response (*TLR4*)4–8. Recently, genome wide association studies (GWAS) have permitted the identification of novel, high prevalence, low penetrance genetic polymorphisms associated with complex human traits, including sporadic cancer risk. Unlike candidate gene approaches, no prior knowledge of the locations of the loci or functions of the gene products is required. Indeed, the mechanism of action of many GWAS-identified genetic variants remains unknown, and the ability to stratify individual cancer risk on the basis of these variants is limited[9].

Recently, the Study Group of the Millennium Genome Project for Cancer published findings of a two-stage GWAS, which demonstrated an association between the rs2294008 single nucleotide polymorphism (SNP) in the *prostate stem cell antigen gene* (*PSCA*), and the risk of gastric cancer in Japanese[10]. The authors subsequently validated the association in a Korean case-control study of gastric cancer. In both the Japanese and Korean study groups, the association was strongest for the diffuse histological-type of gastric cancer (OR = 4.18; 95% CI, 2.88–6.21 for Japanese). The finding of an association between gastric cancer and rs2294008 has now been replicated in several, independent, Asian case-control studies[11–14], and was also confirmed in a recent gastric cancer GWAS in a Chinese population15. Limited functional data exist on the effect of the rs2294008 C>T transition, however results from a reporter assay suggest that the T allele reduces upstream *PSCA* transcriptional activity[10].

Whether the rs2294008 polymorphism confers increased risk of gastric cancer in Caucasian populations has not yet been established. Furthermore, it is not known at what stage in

gastric carcinogenesis the *PSCA* polymorphism exerts its effect. In order to address these questions, we performed a genotyping study that included four independent, Caucasian, case-control studies, comprising one study of gastric cancer, one study of gastric and esophageal cancers, and two studies of chronic atrophic gastritis.

## Materials and Methods

### Study populations

To determine whether the rs2294008 polymorphism is associated with the risk of premalignant change in the stomach, we employed two case-control studies of chronic atrophic gastritis (CAG). In the Scottish Gastric Cancer Relatives Study, a cohort of 166 healthy, Caucasian, first-degree relatives of gastric cancer patients was recruited from the West of Scotland. These subjects have previously been shown to have a high prevalence of hypochlorhydria (pentagastrin-stimulated peak acid output <15mmol$h^{-1}$) in association with *H pylori* infection (assessed by $^{14}$C urea breath test, serology, urease test, culture, and histology) and histological evidence of gastric atrophy[16]. Of the 108 subjects infected with *H pylori*, 43 had hypochlorhydria and gastric atrophy and 65 had normal or high gastric acid secretion. Fifty-eight subjects were free of *H pylori* infection and had normal gastric histology and physiology. One hundred unselected cord blood samples from the West of Scotland were available as population controls, and were used to assess the distribution of alleles at the rs2294008 locus. The two *H pylori*-infected subgroups served as cases and controls for CAG.

The second CAG study group was derived from ESTHER, a large population-based cohort study initiated to investigate new approaches to the early detection and prevention of chronic disease in the elderly[17]. A total of 9,953 participants from Saarland, a federal state in the south-west of Germany, were recruited by their general practitioners during routine health check-ups. At baseline examination, 533 participants without gastric cancer were serologically defined as having CAG by ELISA (Pepsinogen I < 70 ng/ml and pepsinogen I/pepsinogen II ratio < 3)[17–19]. A stratified random sample of 1054 controls was included in the present analysis, with controls frequency-matched to cases by sex and 5-year age group[20,21].

In order to investigate the influence of the rs2294008 polymorphism on upper GI cancer risk in Caucasians, two independent, population-based, case-control studies were utilized. The first was a gastric cancer study derived from a Caucasian population in Warsaw, Poland, in which DNA samples were available from 312 gastric cancer cases (predominantly non-cardia cancers) and 383 randomly-selected population controls, matched by age and sex[22]. The second was a multi-centre esophageal and gastric cancer study conducted in three distinct geographic areas of the United States holding population-based cancer registries[23]; DNA samples were available from 309 gastric cancer cases (123 cardia and 186 non-cardia; 90% Caucasian), 159 esophageal cancer cases (52 squamous cell carcinoma [ESCC] and 107 adenocarcinoma [EAC], 90% Caucasian), and 211 matched population controls (94% Caucasian).

The institutional review boards of the participating centers granted ethical approval for the study, and written informed consent was obtained from all subjects.

### Genotyping

The German CAG study DNA samples were genotyped using Sequenom's MassARRAY® system (Sequenom, San Diego, CA), performing iPLEX® single base primer extension and matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry, as described elsewhere[24]. Genotyping calls were made in real-time with the MassARRAY®

RT software, yielding a call rate of 98% for rs2294008. For the Scottish, Polish and US study groups, DNA samples were genotyped by real-time PCR allelic discrimination using the ABI 7900HT Fast Sequence Detection System (Applied Biosystems, Foster City, CA). A pre-designed TaqMan® SNP genotyping assay was employed, utilizing minor groove binding probes 5′-labeled with VIC or FAM fluorophores to detect the C and T alleles of rs2294008 (Applied Biosystems)25. Genotyping calls were possible in over 99% of samples by analysis of real-time and end-point data. Genotyping results were validated by direct sequencing of selected samples. Real-time PCR allelic discrimination and MALDI-TOF have previously been shown to yield highly comparable, accurate, genotyping results when used in parallel26.

### Statistical Analysis

Hardy-Weinberg equilibrium (HWE) of alleles at the polymorphic locus was assessed by $\chi^2$ statistics. Odds ratios (OR) with Cornfield 95% confidence intervals (CIs) were computed by logistic regression using Statistical Analysis Software (SAS) version 9.1 (SAS Institute Inc., Cary, USA), for the German study data, and STATA version 7.0 software (STATA Press, College Station, TX) for the Scottish, Polish and US study data. ORs for the Scottish study data were adjusted for age and within-family correlation, to account for samples derived from several members of a given family. ORs for the German, Polish, and US studies were adjusted for age and sex. Additional models, performed to adjust for the potential confounding effects of *H pylori* infection, smoking, and, in the US study only, ethnicity, made no material difference to the ORs.

## Results

The alleles at the rs2294008 polymorphic locus were in Hardy-Weinberg equilibrium, with non-significant $\chi^2$ values, in all control populations with the exception of the Polish controls, where there was a heterozygote deficit (HWE $\chi^2 = 6.43$, $p = 0.011$). The frequency of the variant allele in the control populations ranged from 0.369 (Scottish) to 0.518 (Polish).

### The *rs2294008* polymorphism is associated with the risk of gastric cancer precursors

In both the German and Scottish study populations, an association was observed between the risk allele (T) of rs2294008 and CAG (Table 1). For the German study, the OR for individuals carrying one copy of the risk allele was 1.4 (95% CI, 1.1–1.8), whilst for T/T homozygotes, the OR was 1.7 (95% CI, 1.2–2.3). Both inheritance models yielded significant associations, with the higher OR of 1.5 (95% CI, 1.1–1.9) observed in a dominant model. In the Scottish study, the T allele conferred increased risk of atrophy and hypochlorhydria, with the T/T genotype yielding an OR of 4.1 (95% CI, 1.3–12.7) in a recessive model (Table 1). Analysis of the German and Scottish study populations did not reveal an association between rs2294008 and the risk of *H pylori* infection (data not shown).

### The *rs2294008* polymorphism is a risk factor for non-cardia gastric cancer in Caucasians

A positive association was observed between the rs2294008 risk allele and non-cardia gastric cancer in the US study population, and all-site gastric cancer in the Polish study population (Table 2). In the Polish study (where cases were predominantly non-cardia cancers) for all anatomic sub-sites combined, individuals carrying one or two copies of the risk allele had similar ORs of 1.9 (95% CI, 1.2–2.9 and 1.2–3.0 respectively). No association was detected when the small sub-group (n = 36) of cardia cancer cases were analyzed in isolation (data not shown). In keeping with existing literature, the association was strongest for the diffuse histological-type of gastric cancer, with an OR of 3.7 (95% CI, 1.3–12.9) for T/T homozygotes. The association did, however, hold for the intestinal type also, giving an OR of 1.6 (95% CI, 1.0–2.6) in a dominant model.

In the US study population, the association between the rs2294008 risk allele and non-cardia cancer, and diffuse histological-type, was only significant in recessive models, yielding an OR of 1.9 in both cases (95% CI, 1.2–3.0 and 1.1–3.5 respectively). No association was observed in the US study between rs2294008 and intestinal histological-type. Additionally, no relationship was detected between rs2294008 and risk of *H pylori* infection in either the US or Polish study populations (data not shown).

### The *rs2294008* polymorphism is inversely associated with the risk of esophageal and gastric cardia cancers

An inverse association was observed between the rs2294008 polymorphism and proximal cancers of the upper gastrointestinal tract in the US study (Table 2; US study). For gastric cardia cancer, EAC and ESCC, this inverse association appeared strongest for individuals carrying one copy of the risk allele, with ORs of 0.5 (95% CI, 0.3–0.9), 0.5 (95% CI, 0.3–0.9) and 0.4 (95% CI, 0.2–0.8) respectively. Similar associations were obtained for the three proximal cancers in dominant models.

## Discussion

In the present study, we demonstrate that the rs2294008 polymorphism in the *PSCA* gene, previously described as a risk factor for gastric cancer in Asians, is associated with gastric cancer and its precursors (gastric atrophy and hypochlorhydria) in Caucasian populations. In keeping with existing literature, the association is strongest for tumors of diffuse histological-type. We also report the additional novel findings of divergent effects of the polymorphism on cardia and non-cardia gastric cancers, and an inverse association with esophageal cancer of both squamous and adenocarcinoma histotypes.

The main strength of this study is replication of the association with CAG and gastric cancer in multiple independent populations. We acknowledge that the study has potential weaknesses. The limited size of some of the case-control study subgroups increases the chance of over or underestimating the magnitude of effect of the polymorphism. The serological criteria employed for the classification of CAG in the German study have been used extensively in epidemiological studies, and associate with established risk factors for CAG in the ESTHER study population[17]; we cannot, however, exclude bias as a results of serological misclassification of CAG. We recognize that violation of HWE in the Polish controls is an additional potential source of bias. Although the deviation from HWE has likely occurred by chance, it could indicate unexpected population structure, or a systematic genotyping error. Importantly, the same genotyping methodology did not result in deviation from HWE in the US or Scottish controls. Furthermore, numerous previous studies of other markers in this population have not suggested population stratification[4,7,8]. Although intended as a study of Caucasians, the US study did contain a minority of non-Caucasian subjects. Given that we genotyped a single polymorphism, and that functional evidence for rs2294008 derives from only one study[10], we cannot exclude the possibility that the observed associations are partly or entirely due to linkage with another functional polymorphism in the same region.

Our data contain several interesting observations worthy of further consideration. An association between rs2294008 and intestinal histological-type was only detected in the Polish study. One possible reason for this is the limited number of intestinal-type cases in the US study; however one cannot dismiss the possibility that this difference has arisen as a result of population-specific effects mediated through gene-gene or gene-environment interactions[27]. Similarly, background genetic or environmental exposures may account for the differences observed in inheritance models between the US and Polish studies for non-

cardia cancer and diffuse histological-type, although our sample sizes are too small to confidently make such assertions.

The inverse association between rs2294008 and gastric cardia cancer was only observed in the US study; however the number of Polish cardia cases may well have been too small to detect a similar effect. Interestingly, in a recent GWAS in a Chinese population, Abnet *et al.* found no overall association between rs2294008 and gastric cancer[15]. Sub-group analysis, however, revealed a positive association between rs2294008 and non-cardia gastric cancer, yet no association with cardia cancer. A similar dichotomous effect for rs2294008 was observed in the Chinese case-control series of Wu *et al.*[11]. Given that cardia cancer itself appears to be an etiologically heterogeneous condition[28], the opposing effect of rs2294008 on cardia and non-cardia cancer risk observed in our Caucasian population is at least biologically plausible. Interestingly, in per-genotype analyses, the inverse risk for proximal cancers was only significant in C/T heterozygotes. Whilst it is theoretically possible that this reflects a biologically-mediated heterozygote advantage, the numbers of homozygote risk allele cases were small and, since dominant inheritance models gave similar results, it seems more likely that this discrepancy is due to small sample size. Replication of our proximal cancer data in additional populations would help to robustly validate these findings.

Assuming that the divergent effect of the rs2294008 polymorphism on cancer risk at different anatomic sites is genuine, it is not necessarily all that surprising. It is well recognized that proximal and distal upper gastrointestinal cancers have quite different etiologies. The opposing effect may be partly explained in relation to gastric atrophy and hyposecretion. *H pylori* infection and gastric atrophy, risk factors for non-cardia gastric cancer, are inversely associated with reflux esophagitis and EAC[29,30]. One might therefore expect a genetic polymorphism that predisposes to CAG to be inversely associated with EAC. For ESCC, the association may be less easy to explain since recent evidence suggests that an association exists between ESCC, gastric atrophy and hyposecretion, although the association is independent of the severity of gastric atrophy[31]. From a genetic standpoint, similar opposing effects have been described for other GWAS-identified SNPs such as rs6983267, which confers risk for prostate cancer and smoking-related oropharyngeal cancers, but is inversely associated with bladder cancer[32].

The precise function of PSCA *in vivo* is not known. A member of the lymphocyte antigen 6 (Ly-6) superfamily of glycosylphosphatidylinositol (GPI)-anchored cell surface proteins, PSCA was first identified by Reiter *et al.* as a marker overexpressed in a prostate cancer xenograft model33. PSCA expression has subsequently been implicated in prostate cancer stage, Gleason score, and metastatic potential34–36. PSCA has been accorded a somewhat misleading name. Despite its 30% nucleotide homology with stem cell antigen type-2 (SCA-2)[33], another member of the Ly-6 family, PSCA is not a stem cell marker, nor is its expression restricted to prostatic tissue, with PSCA protein expression having been demonstrated in human trophoblast, kidney and stomach10,34. In addition to prostate cancer, *PSCA* appears to be upregulated in a proportion of other human solid tumors including those of the pancreas, urothelium, kidney and ovary[37–40]. In contrast, gastric and esophageal cancers display reduced or absent PSCA expression[10,33,38,41]. Interestingly, *PSCA* also appears to be downregulated in gastric intestinal metaplasia, the precursor lesion of intestinal-type gastric cancer[10]. In keeping with our data, this suggests that PSCA may have a role in the early stages of gastric carcinogenesis. In normal gastric mucosa, PSCA protein expression appears to be localized to dividing and differentiating cells in the isthmus of the gastric mucosal glands[10]. *In vitro* studies have shown that PSCA influences survival in gastric cancer cells, where transfection of *PSCA* into PSCA-negative cells leads to reduced cell proliferation[10]. In contrast, knockdown of *PSCA* in a bladder cancer cell line results in induction of inflammatory gene expression, accompanied by a reduction in cell

grown[42]. The role of PSCA in tumorigenesis therefore appears complex, involving pro-tumorigenic and anti-tumorigenic effects in different contexts[43].

Few functional clues have been gained from the PSCA knock-out mouse, which is viable, fertile, and appears not to be tumor-prone[44]. Insight might better be gleaned by analysis of the functions of other members of the Ly6 family, which have been shown to be involved in differentiation, immune homeostasis and T-cell activation[45,46]. Members of Ly6 protein family bear a structural resemblance to the snake venom α-bungarotoxin, suggesting that they may act as ligands in neuronal signaling[47]. Perhaps the most persuasive functional data specific to PSCA derive from the chick PSCA ortholog. Hruska *et al.* observed that misexpressing PSCA during chick ciliary ganglion development lead to allosteric antagonism of α7 subunit-containing nicotinic acetylcholine receptors (α7-nAChRs), and rescued a neuronal subpopulation from programmed cell death[48]. Interestingly, α7-nAChR-signalling has been implicated in smoking-related carcinogenesis, and α7-nAChRs are required for the COX-II induction observed following exposure of gastric cancer cells to nicotine *in vitro*[49]. It is not known whether PSCA interacts with α7-nAChRs in humans; the related Ly-6 protein, SLURP-1, however, potentiates α7-nAChR signaling in keratinocytes. In addition, mutations in SLURP-1 have been implicated in the inflammatory skin disorder Mal de Meleda, suggesting that SLURP-1 has a role in epidermal homeostasis[50].

The role of nAChR-signaling in non-neuronal cells has become the focus of much attention over recent years, particularly in relation to immune modulation and inflammation. The so-called `cholinergic anti-inflammatory pathway' is exemplified by a rat model where the systemic inflammatory response to endotoxin is reduced by concomitant vagal stimulation[51]. Furthermore, α7 subunit-deficient mice demonstrate an augmented inflammatory response to endotoxaemia due to loss of acetylcholine-mediated inhibition of macrophage TNF release[52].

It is plausible therefore that PSCA might interact with α7-nAChRs in humans, and that the rs2294008 polymorphism may influence the balance of pro-inflammatory and anti-inflammatory signals present in the gastric mucosa. Given that our data implicate PSCA in the development of atrophic gastritis, it is conceivable that PSCA modifies the host inflammatory response to *H pylori* infection. This hypothesis does not exclude potential additional context-specific roles for PSCA in the regulation of epithelial cell proliferation. We therefore suggest that the role of PSCA in pro and anti-inflammatory signaling in humans should be the subject of further scrutiny.

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## Abbreviations used in this paper

| | |
|---|---|
| **CAG** | chronic atrophic gastritis |
| **CI** | confidence interval |
| **EAC** | esophageal adenocarcinoma |
| **ESCC** | esophageal squamous cell carcinoma |

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| **GWAS** | genome wide association study |
| **OR** | odds ratio |
| **PSCA** | prostate stem cell antigen |
| **SNP** | single nucleotide polymorphism |

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**Table 1**

Association of the rs2294008 polymorphism with chronic atrophic gastritis in the German and Scottish studies The Scottish study data are generated by comparison of the two *H pylori*-infected subgroups. Odds ratios, confidence intervals and *p*-values are given for per-genotype, dominant and recessive models.

| | Number of Cases/Controls Odds Ratio[a] (95% Confidence Interval); *p*-value | | | | |
|---|---|---|---|---|---|
| | **CC** | **CT** | **TT** | **Dominant** | **Recessive** |
| German Study | 115/305 1.0 (ref) | 285/542 1.4 (1.1–1.8); 0.012 | 119/189 1.7 (1.2–2.3); 0.001 | 1.5 (1.1–1.9); 0.002 | 1.3 (1.0–1.7); 0.030 |
| Scottish Study | 7/20 1.0 (ref) | 20/38 1.3 (0.6–2.9); 0.570 | 16/7 4.9 (1.3–18.2); 0.018 | 1.8 (0.8–4.1); 0.140 | 4.1 (1.3–12.7); 0.016 |

[a]For the Scottish study, odds ratios were adjusted for age and within-family sampling. For the German study, odds ratios were adjusted for age and sex.

**Table 2**

Association of the rs2294008 polymorphism with upper gastrointestinal cancers in the Polish and US studies. Odds ratios, confidence intervals and *p*-values are given for per-genotype, dominant and recessive models.

| | Number of Cases/Controls Odds Ratio[b] (95% Confidence Interval); *p*-value | | | | |
|---|---|---|---|---|---|
| **Polish Study** | **CC** | **CT** | **TT** | **Dominant** | **Recessive** |
| Gastric Cancer: All sites | 47/101 1.0 (ref) | 143/166 1.9 (1.2–2.9); 0.003 | 102/115 1.9 (1.2–3.0); 0.004 | 1.9 (1.3–2.8); 0.001 | 1.2 (0.9–1.7); 0.184 |
| Diffuse-type | 5/101 1.0 (ref) | 24/166 2.9 (1.0–10.1); 0.028 | 21/155 3.7 (1.3–12.9); 0.008 | 3.2 (1.2–10.7); 0.011 | 1.7 (0.9–3.2); 0.089 |
| Intestinal-type | 35/101 1.0 (ref) | 93/166 1.6 (1.0–2.6); 0.040 | 64/115 1.6 (1.0–2.7); 0.058 | 1.6 (1.0–2.6); 0.029 | 1.2 (0.8–1.7); 0.431 |
| **US Study** | | | | | |
| Gastric Cancer: Non-Cardia | 40/49 1.0 (ref) | 79/110 0.8 (0.5–1.5); 0.521 | 67/49 1.7 (0.9–3.0); 0.069 | 1.1 (0.7–1.8); 0.689 | 1.9 (1.2–3.0); 0.005 |
| Gastric Cancer: Cardia | 45/49 1.0 (ref) | 50/110 0.5 (0.3–0.9); 0.008 | 27/49 0.6 (0.3–1.2) 0.105 | 0.5 (0.3–0.9); 0.010 | 0.9 (0.5–1.6); 0.766 |
| Non-Cardia Diffuse-type | 18/49 1.0 (ref) | 32/110 0.8 (0.4–1.7); 0.493 | 30/49 1.7 (0.8–3.6); 0.155 | 1.1 (0.6–2.1); 0.849 | 1.9 (1.1–3.5); 0.018 |
| Non-Cardia Intestinal-type | 15/49 1.0 (ref) | 25/110 0.7 (0.3–1.7); 0.419 | 18/49 1.2 (0.5–2.9); 0.651 | 0.9 (0.4–1.9); 0.717 | 1.5 (0.7–2.9); 0.246 |
| Esophageal Adenocarcinoma | 40/49 1.0(ref) | 45/110 0.5 (0.3–0.9); 0.012 | 22/49 0.6 (0.3–1.1); 0.072 | 0.5 (0.3–0.9); 0.010 | 0.8 (0.5–1.5); 0.547 |
| Esophageal Squamous Cell | 21/49 1.0 (ref) | 18/110 0.4 (0.2–0.8); 0.007 | 12/49 0.6 (0.2–1.4); 0.174 | 0.4 (0.2–0.9); 0.011 | 1.0 (0.4–2.1); 0.997 |

[b] Odds ratios were adjusted for age and sex.